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A number of different technologies have been deployed to separate, analyze and identify proteins. Typically, identification by mass spectrometry (MS) involves analysis of isolated proteins or peptide fragments, followed by mapping or tandem MS to obtain sequence information. One strategy that has been used to differentiate the resulting spectra involves tagging the proteins with reagents having different masses ("mass tags"). The use of such mass tags allows a number of different samples to be analyzed at the same time and be directly compared.

Paragraph beginning page 14, line 15:

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In one series of preferred embodiments, the PMT reagents comprise biotinylated phenylglyoxals. The dicarbonyl structures in these reagents provide the chemistry for condensation with the guanidine moiety of the arginine side chain. The biotin allows the tagged peptides to be readily separated from the mixture, for example by using a chromatography column. Using a number of different versions of a PMT reagent, each having different masses (e.g., created by different halogen substitutions), allows the protein adducts to be distinguished by mass spectrometry. PMT reagents comprising biotinylated phenylglyoxals can be synthesized from commercially available materials and thus offer rapid and inexpensive access to a diverse set of reagents.

Paragraph beginning page 15, line 7:

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As discussed above, PMT reagents that react with arginine provide broad coverage of the proteome because arginine occurs in proteins with a high relative frequency. Furthermore, because lysine residues can be converted to arginine, these same PMT reagents can also be applied to proteins that contain lysine. The lysine may be derivatized by first converting the  $\epsilon$ -amino group to a guanidine with O-methyl isourea to yield homoarginine. The resultant guanidine group is then condensed, as discussed above, with the phenylglyoxal moiety of the PMT reagent. The chemistry of this modification has been developed to selectively derivatize lysine to homoarginine without the concomitant conversion of the amino-terminus of the peptides. This technique allows assessment of the total arginine and lysine in protein mixtures. Significantly, it also allows the ratio of lysine/arginine to be determined.

Paragraph beginning page 16, line 8:

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The PMT reagent of the present invention can also comprise carboxyl phenylglyoxals (or other substituted di-ketones). A synthetic process for making the unsubstituted carboxyl phenylglyoxals is shown in Figure 5. These dicarbonyl structures not only provide the chemistry for condensation with the guanidine moiety of arginine side chain but also carry mass tags that allow them to be distinguished by mass spectrometry. Tri-substituted benzenoid derivatives carrying four functionalities, while more difficult to synthesize *de novo*, are available commercially and can be readily incorporated into PMT reagents. A phenyl glyoxal derived from 3-carboethoxy 4-hydroxy phenylglyoxal, which is commercially available material, shown in Figure 5 (structure 5).

Paragraph beginning page 16, line 17:

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Referring to Figure 5, the hydroxyl group in structure 5 is first alkylated to yield the intermediate alkoxy phenylglyoxal, the latter being subsequently hydrolyzed to yield the alkoxy substituted carboxy phenylglyoxals ( -OMe and -OEt functioning as the mass tags). A biotin amine can be attached at the carboxyl group to yield the final target. This approach has the advantage of being "modular." That is, the biotin amine serves as the common intermediate to link the different phenylglyoxals or other amino acid reactive moieties.

Paragraph beginning page 16, line 23:

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Commercially available biotin is converted to its active ester form and reacted with 2,2'-(ethylene dioxy) bis (ethylamine). The resulting amino-linked biotin is purified by chromatography and coupled to appropriately substituted carboxy phenylglyoxals to yield PMT reagents of the present invention.

Paragraph beginning page 16, line 31:

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Certain PMT reagents have amino acid reactive moieties that are thiol reactive moieties. Their reaction with cysteine residues yield mass tagged products capable of

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being affinity purified and/or concentrated for mass spectrometric analysis. The reaction of a number of such PMT reagents (comprising biotin moieties) are shown in Figure 7.

Paragraph beginning page 17, line 18:

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In the example shown below, the protein reactive group is fluorescent and also comprises mass tags. In addition to being thiol reactive, the bromobimane moiety is fluorescent. The bromobimane moiety can also be substituted (e.g.,  $R = CH_3, CD_3, C_2H_5, C_2D_5, C_6H_5, C_6D_5$  etc.). Thus, the bromobimane moiety can be the portion of the PMT reagent that is substituted in order to provide mass differentiation. Bromobimane derivatives are commercially available from Molecular Probes (Eugene, Oregon).

Paragraph beginning page 18, line 3:

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The combination of elements in the PMT reagents of the present invention can be accomplished by a large number of possibilities. For example, the recognition moiety (bipyridyl or phenanthroline with metal binding capacity) could be juxtaposed so that the mass tags ( $R = H, CH_3, CD_3, C_2H_5, C_2D_5$ ) are remote from the protein reactive group (phenylglyoxal), as shown below.

Paragraph beginning page 19, line 3:

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An important post-translational modification of proteins is phosphorylation, which occurs predominantly at the OH of serine, threonine and tyrosine residues. PMT reagents may be used according to the methods of the present invention to isolate and quantitate the extent of phosphorylation. The reagents of this invention can be used to capture phosphoproteins (e.g., serine and threonine only) and determine their relative quantities in two or more samples. See Figures 8A and 8B.

Paragraph beginning page 19, line 9:

A14  
In one example, the PMT reagents of the present invention can be applied to perform relative quantification of analytes in two samples using LC-MS/MS and MALDI MS. First, PMT reagents are prepared that are arginine specific, each with a biotin recognition group. These reagents may then be used to test serum samples to address

dynamic range and relative quantification by a number of approaches. For example, proteins in serum can be condensed with PMT reagents and then digested. Alternatively, serum proteins can be digested and then condensed with the PMT reagents. The labeled proteins can then be run through a streptavidin column. LC-MS, MALDI or ESI, can be used to analyze the released biotinylated protein adducts with the PMT reagent.

Paragraph beginning page 27, line 5:

NMR spectra were recorded in a "BRUKER AVANCE-300" (300MHz) instrument and MS was recorded in a "VG-Mass lab Trio-2" quadrupole system.

Paragraph beginning page 28, line 3:

The PMT Target 1 (synthesized as outlined above) was dissolved in 4:1 sodium carbonate buffer (pH=11):DMSO, making a 100 mM solution. 10  $\mu$ L of this solution was then added to 80  $\mu$ L of carbonate buffer (pH = 11) in an EPPENDORF® tube. To this mixture was added 10  $\mu$ L of a 10 mM solution of Angiotensin II (1046.2 g/mol, Sigma) in deionized water. The reaction mixture is vortexed for 1 minute and then placed in a refrigerator at 4°C for 12-15 hours. The reaction mixture was then desalted using ZIPTIP® C-18 P10 (Millipore) and analyzed via MALDI using the following parameters.

In the Drawings:

A drawing sheet with proposed changes to Figure 2 marked in red pursuant to 37 C.F.R. §1.121(d) are being submitted for the Examiner's approval.

REMARKS

Minor amendments have been made to the specification to correct obvious typographical errors. No new matter has been added.